Molecular Cloning of Human Dectin-2

To the Editor:

Murine dectin-2 is a type II C-type lectin receptor and highly associated with dendritic cells (DC), including Langerhans cells (LC) (Ariizumi *et al*, 2000; Bonkobara *et al*, 2001). It bears no signaling motif but is highly homologous (61% identity) to murine DC immunoactivating receptor (DCAR), which acts as an activating receptor through association with Fc receptor γ chain (Kanazawa *et al*, 2003). Its function including the activating capacity, however, has been mostly unknown. Furthermore, it has remained to be clarified whether its human counterpart is really present or not. In this report, we successfully isolated the human orthologue of murine dectin-2 and investigated its mRNA expression profile.

Alignment of a BAC clone of human chromosome 12 (GenBank accession number AC092865) with exons 3 and 4 of murine DCAR provided us the putatively orthologous exons of human clone with appropriate exon–intron boundaries. The full-length cDNA of this human clone was obtained with rapid amplifications of cDNA ends using the cDNA library of human spleen. The contiguous contig is 1613 base pairs in length excluding the poly A tail and contains a putative open reading frame of 627 base pairs (Fig 1*a*). Hydrophobicity profile of the deduced amino acids (Fig 1*b*) demonstrates the presence of a hydrophobic signal anchor of 27 amino acids (underlined in Fig 1*a*), indicating that this molecule is a type II transmembrane protein. Although its short cytoplasmic portion contains no tyrosine residue associated with a signaling motif, its extracellular portion contains six consensus cysteines (circled in Fig 1*a*) forming the carbohydrate recognition domain (CRD). The domain structure of this molecule is shown in Fig 1*b*. Nucleotide sequence alignment with the human chromosome 12 genomic contig (GenBank accession number NT_024397) enabled us to demonstrate the genomic structure of this gene with appropriate exon-intron boundaries. This gene is composed of six exons representing functional domains as shown in Fig 1*c*. These domain and genomic structures are almost the same as those of murine dectin-2 (Ariizumi *et al*, 2000). Genes of this human clone and some related C-type lectins, including DC immunoreceptor (DCIR; Bates *et al*, 1999), C-type lectin-like receptor 6 (CLEC6; GenBank accession number AF411850) and macrophage-inducible C-type lectin (Mincle; Matsumoto *et al*, 1999), are localized on a short region of the human chromosome 12 genomic contig (Fig 1*d*), which is reportedly located on the telomeric end of the natural killer gene complex (Hofer *et al*, 2001). CLEC6 is considered as the human counterpart of murine macrophage-restricted C-type lectin (MCL; Balch *et al*, 1998), because of their high sequence homology (60% identity, data not shown). Comparing the order of these genes with that of the murine counterparts on chromosome 6, the position of this novel human clone is considered exactly syntenic to that of murine dectin-2 (Kanazawa *et al*, 2003).

Overall amino acid sequence of this human clone shows 68% identity with that of murine dectin-2, 52% with murine DCAR and 54% with human BDCA-2 (Fig 1*e*). Although the number of its whole amino acids is the same as that of both murine DCAR and dectin-2, the result of alignment clearly revealed that this human clone represents the putative counterpart of murine dectin-2, rather than DCAR. Positively charged arginine residue in the transmembrane domain (shown by a closed circle in Fig 1*e*), which can partly explain the binding capacity of DCAR with Fc receptor γ chain (Kanazawa *et al*, 2003), is conserved in this human clone and murine dectin-2 (lysine in BDCA-2). The EPN motif in the CRD (shown by plus signs in Fig 1*e*), which may be involved in the binding capacity of mannose-type carbohydrate (Weis et *al*, 1998), is also conserved in this human clone and murine dectin-2 as well as BDCA-2. Collectively, this newly identified human clone has been defined as the human orthologue of murine dectin-2. Although BDCA-2 was proposed to be the human counterpart of murine dectin-2 (Dzionek *et al*, 2001), isolated human dectin-2 is similar but absolutely different from BDCA-2. Considering that the genes of Ly-49 molecules are clustered in the natural killer gene complex of only the murine genome and turn to pseudogenes in human genome (Yokoyama, 1998), presence of distinct human dectin-2 indicates that this molecule have a role in the human immune system.

Furthermore, expression profile of human dectin-2 was examined with RT-PCR. Among normal human tissues, expression of dectin-2 transcript was detected strongly in the lung and weakly in the spleen, lymph nodes, peripheral blood leukocytes, but not observed in the skin (Fig 2). In mouse, dectin-2 mRNA expression was observed in the spleen, thymus (Ariizumi *et al*, 2000), and lung (Fernandes *et al*, 1999), but not in whole skin (Ariizumi *et al*, 2000). Although the expression profile of human dectin-2 is similar to that of the murine counterpart, its expression level in the lung is surprisingly high (Fig 2). It should be further defined what kind of cells in the lung are responsible for this strong expression. Among fractionated peripheral blood cells, human dectin-2 expression was quite strongly observed in purified monocytes and weakly in B cells as well as mononuclear cells (MNC), but not in CD4 and CD8 T cells. After *ex vivo* activation, its expression was reduced in MNC

Abbreviations: CRD, carbohydrate recognition domain; DC, dendritic cells; DCAR, DC immunoactivating receptor; LC, Langerhans cells; MNC, mononuclear cells; moDC, monocyte-derived DC

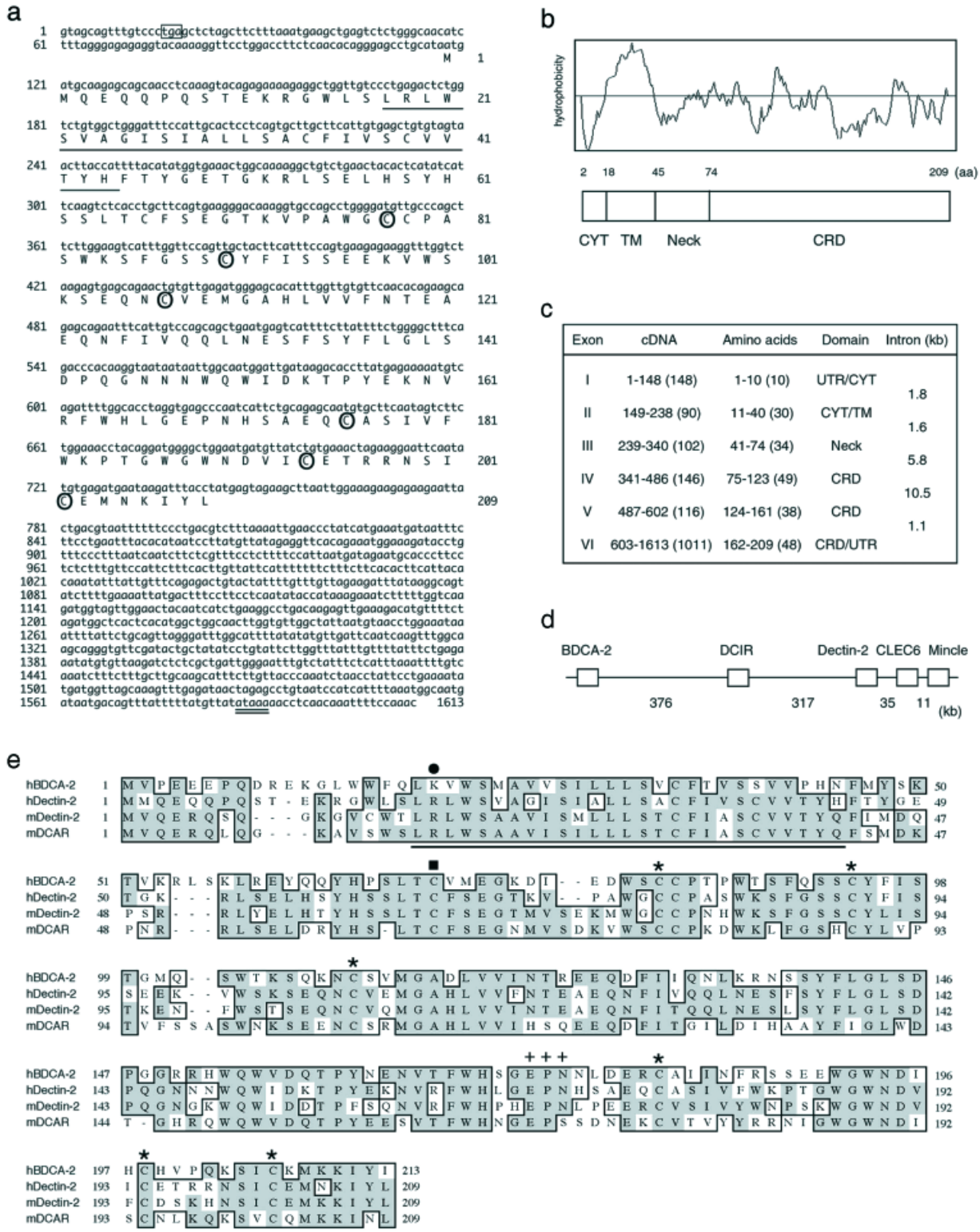

Figure 1

Structures of the human dectin-2 cDNA, protein and gene. (*a*) Nucleotide and predicted amino acid sequences of human dectin-2 cDNA are shown. This nucleotide sequence has been submitted to GenBank under accession number AY321309. The in-flame stop codon upstream of the start codon is surrounded by an open square. The potential transmembrane domain is underlined and the six cysteine residues which are involved in formation of the CRD are circled. The putative poly A additional signal is double underlined. (*b*) Hydrophobicity profile and the domain structure of human dectin-2 protein are shown. GeneWorks software was used for calculation of the hydrophobicity. Numbers of amino acids (aa) represent the junctions of sequential domains. The following abbreviations are used: CYT, cytoplasmic portion; TM, transmembrane domain; UTR, untranslated region. (*c*) Numbers of nucleotides and deduced amino acids of all the exons and introns of human dectin-2 gene are shown. (*d*) Schematic view of the genes of human dectin-2 and related C-type lectins (BDCA-2, DCIR, CLEC6, Mincle) on a short region of human chromosome 12 is shown. Each gene is shown as a box and the distance of sequential genes is indicated by the number of nucleotides (kb: kilobases). (*e*) Deduced amino acid sequence of human dectin-2 was aligned with that of its murine counterpart, human BDCA-2 and murine DCAR using MacVector software. Identical and homologous residues are shaded dark and light, respectively. The potential transmembrane domain is underlined and the conserved positively-charged residue is indicated by a closed circle. The cysteine residue which may be involved in dimer formation is indicated by a closed square and the six conserved cysteine residues in the CRD are shown by asterisks. The EPN motif, which may be involved in the specific recognition of mannose-type sugar, is shown by plus signs.

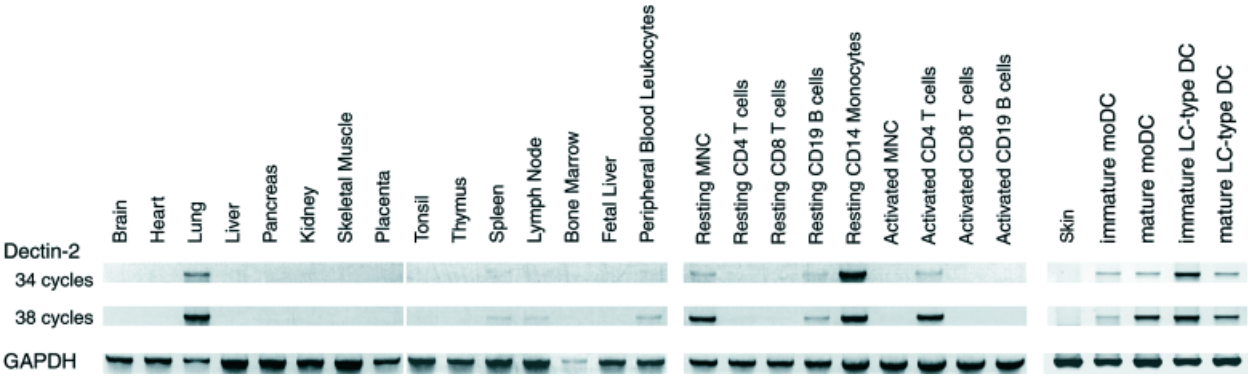

Figure 2
Expression profile of human dectin-2 mRNA. RT-PCR was performed using specific primer pairs of human dectin-2 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) on various human tissues and cell populations. Multiple tissue cDNA panels including Human MTC Panel I, Human Immune System MTC Panel I and Human Blood Fractions MTC Panel were purchased from BD Biosciences Clontech. The other cDNA samples were prepared manually from the normal human skin specimen and *in vitro* generated DC. For generation of immature LC-type DC or moDC, non-adherent fraction of peripheral blood MNC were cultured in the presence of recombinant human granulocyte/macrophage colony-stimulating factor and interleukin-4 with or without transforming growth factor β1 for 7 days, respectively. For generation of LC-type DC, interleukin-4 was added only in the first 2 days. To induce maturation, tumor necrosis factor α, interleukin-6, interleukin-1β and prostaglandin E2 were added into the culture during the last 24 h. The primer pairs used for amplification of human dectin-2 were as follows; 5′-CTG GGC AAC ATC TTT AGG GAG AGA GG-3′ (forward primer) and 5′-GAA TGG AAC AAA GAG AGG AAG GGT GC-3′ (reverse primer). Amplification of 34 cycles or 38 cycles was performed for dectin-2, whereas 30 cycles for GAPDH. Amplified cDNA fragments were separated with 2% agarose gel electrophoresis.

and B cells, whereas interestingly, CD4 T cells turned to produce dectin-2 (Fig 2). Although murine dectin-2 mRNA is reportedly expressed in several hematopoietic cells, such as B cell hybridoma cells (Ariizumi *et al*, 2000), precursor and mature macrophages and mature neutrophils (Fernandes *et al*, 1999), its expression was not recognized in concanavalin A-stimulated T cells (Fernandes *et al*, 1999). Among *in vitro* generated DC, human dectin-2 expression was most strongly observed in immature LC-type DC and very weakly in immature monocyte-derived DC (moDC). After maturation, its expression was upregulated in moDC, but reduced in LC-type DC (Fig 2). Exclusively strong expression of human dectin-2 in immature LC-type DC is quite consistent with the results of the promoter analysis of its murine counterpart (Bonkobara *et al*, 2001). Although the precise expression of human dectin-2 *in vivo* should be further defined, our data suggest its role in activated CD4 T cells as well as in antigen presenting cells, such as monocytes and immature LC.

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References

Ariizumi K, Shen GL, Shikano S, *et al*: Cloning of a second dendritic cell-associated C-type lectin (dectin-2) and its alternatively spliced isoforms. J Biol Chem 275:11957–11963, 2000

Balch SG, McKnight AJ, Seldin MF, Gordon S: Cloning of a novel C-type lectin expressed by murine macrophages. J Biol Chem 273:18656–18664, 1998

Bates EE, Fournier N, Garcia E, *et al*: APCs express DCIR, a novel C-type lectin surface receptor containing an immunoreceptor tyrosine-based inhibitory motif. J Immunol 163:1973–1983, 1999

Bonkobara M, Zukas PK, Shikano S, Nakamura S, Cruz PD, Ariizumi K: Epidermal Langerhans cell-targeted gene expression by a dectin-2 promoter. J Immunol 167:6893–6900, 2001

Dzionek A, Sohma Y, Nagafune J, *et al*: BDCA-2, a novel plasmacytoid dendritic cell-specific type II C-type lectin, mediates antigen capture and is a potent inhibitor of interferon α/β induction. J Exp Med 194:1823–1834, 2001

Fernandes MJ, Finnegan AA, Siracusa LD, Brenner C, Iscove NN, Calabretta B: Characterization of a novel receptor that maps near the natural killer gene complex: Demonstration of carbohydrate binding and expression in hematopoietic cells. Cancer Res 59:2709–2717, 1999

Hofer E, Sobanov Y, Brostjan C, Lehrach H, Duchler M: The centromeric part of the human natural killer (NK) receptor complex: Lectin-like receptor genes expressed in NK, dendritic and endothelial cells. Immunol Rev 181:5–19, 2001

Kanazawa N, Tashiro K, Inaba K, Miyachi Y: Dendritic cell immunoactivating receptor, a novel C-type lectin immunoreceptor, acts as an activating receptor through association with Fc receptor γ chain. J Biol Chem 278:32645–32652, 2003

Matsumoto M, Tanaka T, Kaisho T, *et al*: A novel LPS-inducible C-type lectin is a transcriptional target of NF-IL6 in macrophages. J Immunol 163: 5039–5048, 1999

Weis WI, Taylor ME, Drickamer K: The C-type lectin superfamily in the immune system. Immunol Rev 163:19–34, 1998

Yokoyama WM: Natural killer cell receptors. Curr Opin Immunol 10:298–305, 1998